REMARKS

Reconsideration and withdrawal of the rejections of the claimed invention is respectfully requested in view of the amendments, remarks and enclosures herewith, which place the application in condition for allowance.

I. STATUS OF CLAIMS AND FORMAL MATTERS

Claims 13-15, 21-23, 27, 28, 47-58 and 74 are pending in this application. This amendment is substantially the same as the amendment filed on 10 August 2007 with the differences primarily being additional arguments being presented below. No new matter has been added by this amendment.

It is submitted that the claims, herewith and as originally presented, are patentably distinct over the prior art cited in the Office Action, and that these claims were in full compliance with the requirements of 35 U.S.C. § 112. The amendments of the claims, as presented herein, are not made for purposes of patentability within the meaning of 35 U.S.C. §§§§ 101, 102, 103 or 112. Rather, these amendments and additions are made simply for clarification and to round out the scope of protection to which Applicants are entitled.

II. THE 35 U.S.C. 103(a) REJECTION HAS BEEN OVERCOME

Claims 13-15, 21-23, 27, 28, 47-58 and 74 were rejected as allegedly being obvious by Ruegg et al (US 6180563). The applicants request reconsideration of this rejection for the following reasons.

Background

The applicants maintain their position from the previous office action, but since this was unpersuasive, the applicants will try to present the argument in a different manner to hopefully better explain why the applicants believe the obviousness rejection to be in error.

By apparently fixating on finding the specific compounds of (A) and (B) within Ruegg, the rejection has lost sight of what is the applicants' invention when considering the applicants' invention and Ruegg as a whole.

While it is true that the use of the term "comprising" could conceivably encompass trifloxysulfuron, this is not the inventive step of the applicants' claimed invention, i.e., it is irrelevant what other compounds are encompassed by the term "comprising" so long as the combination of compounds of formula (A) with herbicides (B1) metolachlor, (B2) bispyribac or

its salts, and pyrithiobac or its salts; or (B3) sethoxydim and clethodim produce synergistic effects in combatting harmful plants in cotton crops.

As stated in MPEP 2142 (Legal Concept of *Prima Facie* Obviousness) - "To reach a proper determination under 35 U.S.C. 103, the examiner must step backward in time and into the shoes worn by the hypothetical "person of ordinary skill in the art" when the invention was unknown and just before it was made. In view of all factual information, the examiner must then make a determination whether the claimed invention "as a whole" would have been obvious at that time to that person. Knowledge of applicant's disclosure must be put aside in reaching this determination, yet kept in mind in order to determine the "differences," conduct the search and evaluate the "subject matter as a whole" of the invention."

Applying this concept to the facts of this case, the Examiner is stepping into the shoes of the person of ordinary skill in the art ***just before the applicants' invention was made***. When presented with the Ruegg reference, there would be a determination of what would be obvious variations of this invention and what does this reference teach as a whole.

Discussion regarding synergistic effects

When reading this reference without the benefit of the applicants' claims, it is clear that Ruegg is directed toward the use of glyphosate/glufosinate in combination with sulfonylureas (compound (I)) to produce synergistic effects. Other herbicides may be added to the combination, ***so long as the combination of glyphosate/glufosinate in combination with sulfonylurea produce synergistic effects***. Nowhere in the disclosure of Ruegg is it suggested that combining glyphosate/glufosinate with other herbicides such as (B1) metolachlor, (B2) bispyribac or its salts, and pyrithiobac or its salts; or (B3) sethoxydim and clethodim would produce synergistic effects in combatting harmful plants in cotton crops.[1]

The Office Action appears to acknowledge that Ruegg does not teach synergistic effects for the combinations claimed by the applicants by acknowledging that the applicants' did

[1] As a test of the Examiner's assertion of obviousness, the applicants suggest showing the Ruegg reference to a colleague who has never seen the applicants' claims before and ask them what they would consider to be obvious variants of the Ruegg reference after reading it without benefit of the applicants' claims. While they may suggest that additional herbicides could be added to the glyphosate/glufosinate with sulfonylurea combination, it is highly unlikely that they would glean that glyphosate/glufosinate + (B1) metolachlor, (B2) bispyribac or its salts, and pyrithiobac or its salts; or (B3) sethoxydim and clethodim would produce synergistic effects especially in the absence of any evidence supporting synergy based on this combination.

provide "...unexpected results for the above combination of ingredients as shown on pages 31-35 of the specification." (page 3, 3rd and 4th line from bottom of page). However, the unexpected results were not given their proper weight as it was alleged that the unexpected results are for specific application rates of each herbicide, i.e. there is no basis or evidence provided which supports the position that specific application rates are a necessary element of the claim or is not within the practicable skill of those in the art. As noted by in the Office Action, Ruegg recited broad application rates in their specification (which was not necessary to be included in their claims) for their own combination of herbicides and it is clear that some amount of experimentation with application rate parameters is permitted within the art once it is known which herbicides to select.

The key to the invention which Ruegg does not teach is the combination of glyphosate/glufosinate in combination with sulfonylurea produce synergistic effects; only the applicants provide this teaching. However, once provided with this teaching, the skilled artisan is more than capable of determining the appropriate application rate absence evidence to the contrary.

Interpretation of Data is Incorrect

With regard to the conclusion that combining substances of the same utility with the expectation of at least an additive effect is incorrect. The additive effect is only a theoretical maximum which presumes that there is no competition between the substances to produce the effect or that no detrimental changes occur which might decrease the additive effect.[2]

Contrary to the assertion in the Office Action, an improvement according to the "additive method" is indicative of a clear synergism, even if the effect is only slightly above the level of the formal addition of the effects of the single active ingredients.

The latter conclusion is reliable because the herbicidal effect of a combination of two herbicides up to formal addition of the effects obtained by the single application of each active ingredient only is not what is expected by the person skilled in art.

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[2] As an illustration of this concept, think of an enzymatic reaction where Enzyme A and Enzyme B, which recognize the same active site, both have an activity of 50% on a substrate when used alone. Adding Enzyme A and Enzyme B to the substrate would still result in 50% activity (not 100%) because they would be competing for the same active site. Likewise, the according to the logic of the Office Action, adding two herbicides which each have 100% activity, would result in the non-sensical conclusion that the resulting composition would have 200% activity.

Synergism requires a showing of more than an additive effect. Not only have the applicants shown this, the applicants have shown synergism even when assuming a perfect additive effect. Moreover, the Examiner is mistaken in asserting that small differences between theoretical maximum additive effects and the effects actually shown are not evidence of synergism. This is synergism on its face. In addition, at high levels of activity, even small increases beyond the theoretical maximum are surprising evidence of synergism.

For example, the effect of 450 g/ha glufosinate-ammonium (A1.2) and 930 g/ha metolachlor (B1.9) as shown in Table 2 (see page 32 of the specification) produced an effect which was 6% greated that the theoretical maximum additive effect (i.e. 94% vs. 88%). This effect is surprising because the effect of glufosinate-ammonium alone at 450 g/ha produces 0% effect.

Other measures of synergism such as the Colby equation (see page 28 of the specification)[3] show lesser expectations of additive effects. For example, when assuming that glufosinate-ammonium (A1.2) had some herbicidal effect (e.g. 5%), this and 930 g/ha metolachlor (B1.9) still had 88% activity, the additive effect is not 93% (5% + 88%) but only 88.6%. (E = 5% + 88% - (5 x 88/100)% = 88.6%)

Likewise, in Table 5, the example with testing the combination of 400 g/ha glufosinate-ammonium (A1.2) + 105 g/ha pyrithiobac (B2.4) produces a herbicidal effect of 95% on Ipomoea, i.e. 10% above the effect obtained by calculation of the formal addition of the effects 43% + 42% = 85%. This increase of 10% action is a remarkable increase especially if compared with the expected effect of Colby which is calculated as follows:

$$E = 43\% + 42\ \% - (43\cdot 42/100)\ \% = 85\% - 18{,}2\% = 66.8\ \%$$

Compared to expected effect calculated according to the Colby equation the increase up to 85% is thus an increase of more than 18 points, i.e. 27% more based on the expected effect of 66,8% (18.2:66.8 x 100% = 27.2%).

The combination effect is thus clearly synergistic.

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[3] $E = A+B-(A\cdot B/100)$

A, B = effect of a. i. A or B in % at a or b g a.i./ha, respectively;
E = expected effect of the combination in % at a+b g a.i./ha.

The Office Action appears to discount the evaluation method of Colby (see *Weeds* 15 (1967) S. 20 to 22 - copy provided with this office action and which was mentioned in the "Biological Examples" section of the specification), but offers no evidence which contradicts the finds from this peer-reviewed journal. The treatise of Colby and detailed findings therein makes it clear that the formal addition of herbicidal effects of the herbicidal components of a mixture cannot be expected for scientific reasons absent evidence to the contrary. The expected effect will always be below the formal addition of the herbicidal effects.

Lastly, while the applicants have provided evidence in support of synergism, there has been no countervailing evidence presented in any office action; only an unsupported opinion with regard to the applicants' evidence.

Therefore, Ruegg does not teach the inventive concept of the applicants claimed invention and the applicants have shown evidence of synergy and as such the applicants' claims are unobvious over Ruegg.

CONCLUSION

In view of the remarks and amendments herewith, the application is believed to be in condition for allowance. Favorable reconsideration of the application and prompt issuance of a Notice of Allowance are earnestly solicited. The undersigned looks forward to hearing favorably from the Examiner at an early date, and, the Examiner is invited to telephonically contact the undersigned to advance prosecution.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: Howard C. Lee

Marilyn M. Brogan   Howard C. Lee
Reg. No. 31,223   Reg. No. 48,104
Telephone: (212) 588-0800
Facsimile: (212) 588-0500

2. Frans, R. E. 1961. Preplanting herbicides for controlling Johnsongrass in field crops. Proc. SWC 14:37–13.
3. Freeman, J. F. and T. W. Waldrep. 1963. Combination treatments of dalapon grass killer preplow and EPTC preemergence for control of Johnsongrass in corn. Down to Earth 18(1):20–23. 1963.
4. Hauser, Ellis W., J. T. Thompson, and S. V. Stacy. 1955. The effect of chemicals on the control of Johnsongrass and nutgrass with and without disking. Proc. SWC 8:399–104.
5. Pammel, L. H. and C. M. King. 1919. Johnsongrass as a weed in Southwestern Iowa. Iowa Agr. Expt. Sta. Cir. 25. 4 p.
6. Rea, H. E. 1955. The effectiveness of dalapon in controlling Johnsongrass. Proc. SWC 8:391–396.
7. Ryder, Gordon J. and C. J. Willard. 1956. You can control Johnsongrass. Ohio Agr. Ext. Serv. Bull. 342.
8. Watson, A. J. 1954. Field performance of dalapon, a new grass controlling herbicide. Proc. SWC 7:200–204.

Calculating Synergistic and Antagonistic Responses of Herbicide Combinations[1]

S. R. Colby[2]

Abstract. The responses of herbicides applied singly are used in calculating the "expected" response when they are combined. The expected response for a combination is obtained by taking the product of the percent-of-control values for herbicides applied alone and dividing by $(100)^{n-1}$ where n is the number of herbicides in the combination.

In spite of the tremendous increase in testing of herbicide combinations, the words "synergistic" and "antagonistic" have been largely avoided in publication of results. Uncertainty in determining "expected" responses for herbicide combinations may be partially responsible for the failure of workers to report synergism and antagonism. Another difficulty frequently encountered is that the herbicides used in combination are not applied singly in the same study. When herbicides have not been applied singly, there is no basis for predicting the response when they are applied in combination.

Several mathematical methods are available for testing the additivity of herbicide combinations (3, 6). This paper presents a method which facilitates calculating "expected" responses of herbicide combinations. The "expected" response for a given combination of two herbicides can be calculated as follows (3, 5):

If X = the percent inhibition of growth by herbicide A at p lb/A
and Y = the percent inhibition of growth by herbicide B at q lb/A
and E = the expected percent inhibition of growth by herbicides A + B at p + q lb/A
then, according to Gowing (3):

$$E = X + \frac{Y\,(100-X)}{100} \qquad \text{(I)}$$

Algebraic manipulation of terms in equation I yields equation II, the form used by Limpel *et al.* (5):

$$E = X + Y - \frac{XY}{100} \qquad \text{(II)}$$

When the observed response is greater than expected, the combination is synergistic; when less than expected, it is antagonistic. If the observed and expected responses are equal, the combination is additive.

In the use of equation II, original units of data, such as weed counts or fresh or dry weights of plants, are converted to "percent inhibition" values. Once this is done, it is necessary to perform one addition, a subtraction, a multiplication, and one division to obtain each expected response (equation II).

If instead, we convert the original data to "percent-of-control" values, the number of arithmetic operations required to obtain "E" is reduced.

Let X_1 = growth as a percent-of-control with herbicide A at p lb/A
and Y_1 = growth as a percent-of-control with herbicide B at q lb/A
and E_1 = expected growth as a percent-of-control with herbicides A + B at p + q lb/A

then $E_1 = 100 - E$
$X_1 = 100 - X$
$Y_1 = 100 - Y$

$$\text{hence } E_1 = 100 - \left(X + Y - \frac{XY}{100}\right)$$

$$\text{and } E_1 = 100 - \left((100-X_1) + (100-Y_1) - \frac{(100-X_1)(100-Y_1)}{100}\right)$$

$$\text{finally } E_1 = \frac{X_1 Y_1}{100} \qquad \text{(III)}$$

The use of formula III as compared with formula II eliminates the addition and subtraction, thus reducing the number of operations required to obtain an "expected" response.

Colby (2) extended formula I to apply to three-way combinations.

Thus, if Z = the percent inhibition of growth by herbicide C at r lb/A

$$\text{then } E = X + Y + Z - \frac{(XY + XZ + YZ)}{100} + \frac{XYZ}{10{,}000} \qquad \text{(IV)}$$

[1]Received for publication April 11, 1966. Contribution No. 3796 and Scientific Article No. A 1271 of the Maryland Agricultural Experiment Station, Department of Agronomy, University of Maryland, College Park.

[2]Assistant Professor, Agronomy Department, University of Maryland, College Park.

Now if Z_1 = growth as a percent-of-control with herbicide C at r lb/A

$$\text{then } E_1 = \frac{X_1Y_1Z_1}{10,000} \qquad (V)$$

Obviously, the use of formula V instead of IV reduces the number of arithmetic operations required to obtain the expected response since the subtractions and additions are eliminated. In general, the expected response for any combination of herbicides may be obtained by taking the product of the percent-of-control values for herbicides applied alone and dividing by $(100)^{n-1}$ where n is the number of herbicides in the combination. Each herbicide must be applied singly at the same rate as used in combination.

Data published by Jagschitz and Skogley (4) are used to illustrate the calculation of expected responses for herbicide combinations. Four herbicides were applied singly and in certain combinations for the control of several weeds in turfgrass. The data as originally presented have been converted to percent-of-control values and are shown in Tables 1 and 2. Expected values for the combinations are shown in

Table 1. Dandelion control in fairway turf treated with various herbicides October 8, 1964.[a]

Herbicide	lb/A	Dandelion response, %-of-control, 10-7-65[b]		
dicamba	0.125	55		
	0.25	25		
	0.5	43		
mecoprop	0.5	97		
	1.0	81		
	1.5	79		
2,4-D	0.5	63		
	1.0	54		
	1.5	44		
picloram	0.0625	40		
	0.25	10		
dicamba + mecoprop	0.125 + .5	51	(53)	+2
	0.125 + 1.0	33	(45)	+12
dicamba + 2,4-D	0.125 + 0.5	51	(35)	−16
	0.125 + 1.0	64	(30)	−34
	0.25 + 1.0	58	(14)	−44
	0.5 + 1.0	46	(23)	−23
mecoprop + 2,4-D	0.5 + 0.5	56	(61)	+5
	0.5 + 1.0	47	(52)	+5
	1.0 + 0.5	43	(51)	+8
	1.0 + 1.0	57	(44)	−13
	1.5 + 1.0	76	(43)	−33
dicamba + mecoprop + 2,4-D	0.125 + 0.5 + 0.5	63	(34)	−29
	0.125 + 1.0 + 0.5	31	(28)	−3
	0.125 + 0.5 + 1.0	52	(29)	−23
	0.125 + 1.0 + 1.0	54	(24)	−30
dicamba + mecoprop + 2,4-D + picloram	0.125 + 0.5 + 0.5 + 0.0625	77	(13)	−64

[a]Adapted from the data of Jagschitz and Skogley (4).
[b]Expected responses for combinations are shown in parentheses following each observed response. The differences between observed and expected values also are shown by a plus sign to indicate synergism and a minus, antagonism.

parentheses following each observed value. To the right of each expected value, the difference between observed and expected values is shown. A positive value is indicative of a synergistic response while a negative value is indicative of an antagonistic response. If the observed and expected values had been computed individually for each replication, then a chi-square test could have been used to determine the statistical significance of the differences between observed and expected values. Even without the chi-square test, several conclusions seem probable from the data in Tables 1 and 2. First, the combinations appear antagonistic on dandelion. Furthermore, the antagonism seems to be greater with increasing combined rates, especially when the herbicides were applied in 1964. Possibly this antagonism is caused by greater contact injury or more plant tops being killed at higher rates resulting in less translocation of herbicide into the dandelion roots. It also appears from Table 2 that different weeds respond differently to the same

Table 2. Chickweed and dandelion control in fairway turf treated with various herbicides May 25, 1965.[a]

Herbicide	lb/A	Chickweed response, %-of-control, 10-19-65[b]			Dandelion response, %-of-control, 10-19-65[b]		
dicamba	0.125	40			66		
	0.25	1			53		
	0.5	0			49		
Mecoprop	0.5	16			87		
	1.0	0			62		
	1.5	0			72		
2,4-D	0.5	51			75		
	1.0	32			64		
	1.5	71			36		
dicamba + mecoprop	0.125 + .5	1	(6)	+5	70	(57)	−13
	0.125 + 1.0	0	(0)		28	(41)	+13
dicamba + 2,4-D	0.125 + .5	9	(20)	+11	67	(50)	−17
	0.125 + 1.0	3	(13)	+10	21	(42)	+21
	0.25 + 1.0	0	(3)	+3	41	(34)	−7
	0.5 + 1.0	0	(0)		53	(31)	−22
mecoprop + 2,4-D	0.5 + .5	1	(8)	+7	77	(65)	−12
	0.5 + 1.0	1	(5)	+4	68	(56)	−12
	1.0 + 0.5	1	(0)	−1	70	(47)	−23
	1.0 + 1.0	1	(0)	−1	55	(40)	−15
	1.5 + 1.0	1	(0)	−1	69	(46)	−23
dicamba + mecoprop + 2,4-D	0.125 + 0.5 + 0.5	1	(3)	+2	57	(43)	−14
	0.125 + 1.0 + 0.5	0	(0)		64	(31)	−33
	0.125 + 0.5 + 1.0	0	(2)	+2	54	(37)	−17
	0.125 + 1.0 + 1.0	0	(0)		29	(26)	−3

[a]Adapted from the data of Jagschitz and Skogley (4).
[b]Expected responses for combinations are shown in parentheses following each observed response. The differences between observed and expected values also are shown by a plus sign to indicate synergism and a minus, antagonism.

combination. Thus, combinations which were about additive or possibly synergistic on chickweed were antagonistic, in general, on dandelion.

The calculations involved in determining the expected response of one three-way combination from Table 1 illustrate the efficiency of formula V compared to formula IV. For example, using dicamba at 0.125 lb/A in combination with mecoprop at 0.5 lb/A and 2,4-D at 0.5 lb/A the expected response is calculated as follows using formula IV and the data in terms of percent weed control as originally reported by Jagschitz *et al.* (4).

$$E = 45 + 3 + 37 - \frac{(45(3) + 45(37) + 3(37))}{100} + \frac{(45)\,(3)\,(37)}{10,000}$$

$$= 85 \quad \frac{(135 + 1665 + 111)}{100} + \frac{4995}{10,000}$$

$$= 85 \quad 19.11 + 0.50$$

$$= 66.39\% \text{ weed control expected}$$

Using formula V and percent-of-control values, the computation is

$$E_1 = \frac{(55)\ (97)\ (63)}{10{,}000}$$

$= 33.61$ percent-of-control

and 33.61%-of-control is equal to 66.39% weed control.

Obviously, there are practical limitations in using mathematical formulas in predicting the responses for herbicide combinations. The methods described here are approximations, but they represent an improvement over no attempt to predict responses. The computations described should most effectively be applied to populations of single species although this would not seem to be an absolute requirement. Furthermore, the formulas are most accurate when values of X, Y, and Z are near the 50% level since the dose-response curves deviate least from linearity at the 50% level.

Literature Cited

1. Colby, S. R. and G. F. Warren. 1963. Herbicides: combination enhances selectivity. Science 141:3578.
2. Colby, S. R. 1965. Greenhouse evaluation of herbicide combinations. Proc. NEWCC 19:312–320.
3. Gowing, D. P. 1960. Comments on tests of herbicide mixtures. Weeds 8:379–391.
4. Jagschitz, J. A. and C. R. Skogley. 1966. Dicamba, mecoprop and 2,4-D combinations for the control of clover, chickweed and dandelion in turfgrass. Proc. NEWCC 20:496–501.
5. Limpel, L. E., P. H. Schuldt, and D. Lamont. 1962. Weed control by dimethyl tetrachloroterephthalate alone and in certain combinations. Proc. NEWCC 16:48–53.
6. Tammes, P. M. L. 1964. Isoboles, a graphic representation of synergism in pesticides. Neth. J. Plant. Path. 70:73–80.

Seasonal Variation in Sprouting and Available Carbohydrate in Yellow Nutsedge Tubers[1]

R. B. Taylorson[2]

Abstract. Two morphological types of tubers of yellow nutsedge (*Cyperus esculentus* L.) were collected over a 2-year period and were sprouted in the laboratory. Tuber dormancy occurred during late summer and early fall. Sprouting was highest during the winter and spring. Mechanical disturbance of the nutsedge stand increased tuber sprouting. Available carbohydrates followed a pattern similar to sprouting: minimum levels were found during late summer. The two types of tubers appeared to be similar in respect to the characteristics studied.

Introduction

Dormancy commonly occurs in various organs and at different seasons of the year among species of higher plants (7). Generally, little is known of dormancy in subterranean organs of weeds, including tubers of yellow nutsedge (*Cyperus esculentus* L.). Tumbleson and Kommedahl (6) indicated that tubers were dormant when dug in September but would germinate in June. Breaking of tuber dormancy was thought to be associated with low temperature and leachable inhibitors. Other research has dealt mainly with methods of breaking dormancy of tubers by chemical techniques (2). Control of yellow nutsedge with postemergence herbicides is partially dependent on tuber dormancy, since emergence of shoots must be optimum when the herbicides are applied for maximum effects.

Another factor often related to effectiveness of herbicides in the control of perennial weed species is the level of reserve carbohydrates. However, studies attempting to relate carbohydrate levels with herbicide susceptibility have not been clearly successful (4, 5).

In these studies, I have attempted to characterize tuber dormancy and carbohydrate content and their possible relation to herbicide utilization.

Methods and Materials

A dense stand of yellow nutsedge growing in a field of Tifton loamy sand was the source of plant material. Samples were collected at monthly intervals from July, 1962 to June, 1964. During July, 1962 to June, 1963, samples were randomly collected over the infested area. The stand was not disturbed mechanically except for an early spring plowing and harrowing. During the months of July to November, 1963, the area was subdivided into 25 by 50 ft plots. Three twice-replicated treatments were imposed. One treatment was a continuation of the mechanically undisturbed stand mentioned above. Other treatments were (a) mowing approximately 2 weeks prior to the next sampling date and (b) disk-harrowing approximately 2 weeks prior to the next sampling date. At the conclusion of this sampling period, further collections were made only from the mechanically undisturbed plots.

At each sampling date, duplicate lots of approximately 500 tubers were recovered by working the soil through a coarse screen. On several occasions during 1963, unwashed tubers were recovered from mechanically undisturbed plot samples by searching the soil samples and brushing off most of the adhering soil from the tubers.

Except for the unwashed lots, the tubers were subjectively graded into four types according to external color and morphology, and then counted. Type A tubers were black-skinned, shriveled, and usually dead; type B were black but turgid; type C were brown and turgid;

[1]Received for publication February 24, 1966. Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and the Coastal Plain Experiment Station, Tifton, Georgia.

[2]Plant Physiologist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Tifton, Georgia. Present address is Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

Calculating Synergistic and Antagonistic Responses of Herbicide Combinations[1]

S. R. Colby[2]

Abstract. The responses of herbicides applied singly are used in calculating the "expected" response when they are combined. The expected response for a combination is obtained by taking the product of the percent-of-control values for herbicides applied alone and dividing by $(100)^{n-1}$ where n is the number of herbicides in the combination.

In spite of the tremendous increase in testing of herbicide combinations, the words "synergistic" and "antagonistic" have been largely avoided in publication of results. Uncertainty in determining "expected" responses for herbicide combinations may be partially responsible for the failure of workers to report synergism and antagonism. Another difficulty frequently encountered is that the herbicides used in combination are not applied singly in the same study. When herbicides have not been applied singly, there is no basis for predicting the response when they are applied in combination.

Several Mathematical methods are available for testing the additivity of herbicide combinations (3, 6). This paper presents a method which facilitates calculating "expected" responses of herbicide combinations. The "expected" response for a given combination of two herbicides can be calculated as follows (3, 5):

If X = the percent inhibition of growth by herbicide A at p lb/A
and Y = the percent inhibition of growth by herbicide B at q lb/A
and E = the expected percent inhibition of growth by herbicides A + B at p + q lb/A

then, according to Gowing (3):

$$E = X + \frac{Y(100 - X)}{100} \qquad \text{(I)}$$

Algebraic manipulation of terms in equation I yields equation II, the form used by Limpel *et al.* (5):

$$E = X + Y - \frac{XY}{100} \qquad \text{(II)}$$

When the observed response is greater than expected, the combination is synergistic; when less than expected, it is antagonistic. If the observed and expected responses are equal, the combination is additive.

In the use of equation II, original units of data, such as weed counts or fresh or dry weights of plants, are converted to "percent inhibition" values. Once this is done, it is necessary to perform one addition, a subtraction, a multiplication, and one division to obtain each expected response (equation II).

If instead, we convert the original data to "percent-of-control" values, the number of arithmetic operations required to obtain "E" is reduced.

Let X_1 = growth as a percent-of-control with herbicide A at p lb/A
and Y_1 = growth as a percent-of-control with herbicide B at q lb/A
and E_1 = expected growth as a percent-of-control with herbicides A + B at p + q lb/A

then E_1 = 100 - E
X_1 = 100 - X
Y_1 = 100 - Y

hence $$E_1 = 100 - \left(X + Y - \frac{XY}{100}\right)$$

and $$E_1 = 100 - \left[(100 - X_1) + (100 - Y_1) - \frac{(100 - X_1)(100 - Y_1)}{100}\right]$$

finally $$E_1 = \frac{X_1 Y_1}{100} \qquad \text{(III)}$$

The use of formula III as compared with formula II eliminates the addition and subtraction, thus reducing the number of operations required to obtain an "expected" response.

Colby (2) extended formula I to apply to three-way combinations

Thus, if Z = the percent inhibition of growth by herbicide C at r lb/A

then $$E = X + Y + Z - \frac{(XY + XZ + YZ)}{100} + \frac{XYZ}{10{,}000} \qquad \text{(IV)}$$

Now if Z_1 = growth as a percent-of-control with herbicide C at r lb/A

then $$E_1 = \frac{X_1 Y_1 Z_1}{10{,}000} \qquad \text{(V)}$$

Obviously, the use of formula V instead of IV reduces the number of arithmetic operations required to obtain the expected response since the subtractions and additions are eliminated. In general, the expected response for any combination of herbicides may be obtained by taking the product of the percent-of-control values for herbicides applied alone and dividing by $(100)^{n-1}$ where n is the number of herbicides in the combination. Each herbicide must be applied singly at the same rate as used in combination.

Data published by Jagschitz and Skogley (4) are used to illustrate the calculation of expected responses for herbicide combinations. Four herbicides were applied singly and in certain combinations for the control of several weeds in turfgrass. The data as originally presented have been converted to percent-of-control values and are shown in Tables 1 and 2. Expected values for the combinations are shown in parentheses following each observed value. To the right of each expected value, the difference between observed and expected values is shown. A positive value is indicative of a synergistic response while a negative value is indicative of an antagonistic response. If the observed and expected values had been computed

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[2] Assistant Professor, Agronomy Department, University of Maryland, College Park.

Table 1. Dandelion control in fairway turf treated with various herbicides October 8, 1964.[a]				
Herbicide	lb/A	Dandelion response, %-of-control, 10-7-65[b]		
dicamba........................	0.125	55		
	0.25	25		
	0.5	43		
mecoprop................	0.5	97		
	1.0	81		
	1.5	79		
2,4-D.....................	0.5	63		
	1.0	54		
	1.5	44		
pieloram..................	0.0625	40		
	0.25	10		
dicamba + mecoprop...	0.125 + 0.5	51	(53)	+2
	0.125 + 1.0	33	(45)	+12
dicamba + 2, 4-D.......	0.125 + 0.5	51	(35)	-16
	0.125 + 1.0	64	(30)	-34
	0.25 + 1.0	58	(14)	-44
	0.5 + 1.0	46	(23)	-23
mecoprop + 2, 4-D......	0.5 + 0.5	56	(61)	+5
	0.5 + 1.0	47	(53)	+5
	1.0 + 0.5	43	(51)	+8
	1.0 + 1.0	57	(44)	-13
	1.5 + 1.0	76	(43)	-33
dicamba + mecoprop + 2, 4-D....................	0.125+0.5+0.5	63	(34)	-29
	0.125+1.0+0.5	31	(28)	-3
	0.125+0.5+1.0	53	(29)	-23
	0.125+1.0+1.0	54	(24)	-30
dicamba + mecoprop + 2, 4-D + pieloram.......	0.125 + 0.5 + 0.5 + 0.0625	77	(13)	-64

[a]Adapted from the data of Jagschitz and Skogley (4).
[b]Expected responses for combinations are shown in parentheses following each observed response. The differences between observed and expected values also are shown by a plus sign to indicate synergism and a minus, antagonism.

individually for each replication, then a chi-square test could have been used to determine the statistical significance of the differences between observed and expected values. Even without the chi-square test, several conclusions seem probable from the data in Tables 1 and 2. First, the combinations appear antagonistic on dandelion. Furthermore, the antagonism seems to be greater with increasing combined rates, especially when the herbicides were applied in 1964. Possibly this antagonism is caused by greater contact injury or more plant tops being killed at higher rates resulting in less translocation of herbicide into the dandelion roots. It also appears from Table 2 that different weeds respond differently to the same combination. Thus, combinations, which were about additive or possibly synergistic on chickweed were antagonistic, in general, on dandelion.

Table 2. Chickweed and dandelion control in fairway turf treated with various herbicides May 25, 1965.[a]							
Herbicide	lb/A	Chickweed response, %-of-control, 10-19-65[b]			Dandelion response, %-of-control, 10-19-65[b]		
dicamba........................	0.125	40			66		
	0.25	1			53		
	0.5	0			49		
mecoprop................	0.5	16			87		
	1.0	0			62		
	1.5	0			72		
2,4-D.....................	0.5	51			75		
	1.0	32			64		
	1.5	71			36		
dicamba + mecoprop...	0.125 + 0.5	1	(6)	+5	70	(57)	-13
	0.125 + 1.0	0	(0)		28	(41)	+13
dicamba + 2, 4-D.......	0.125 + 0.5	9	(20)	+11	67	(50)	-17
	0.125 + 1.0	3	(13)	+10	21	(42)	+21
	0.25 + 1.0	0	(3)	+3	41	(34)	-7
	0.5 + 1.0	0	(0)		53	(31)	-22
mecoprop + 2, 4-D......	0.5 + 0.5	1	(8)	+7	77	(65)	-12
	0.5 + 1.0	1	(5)	+4	68	(56)	-12
	1.0 + 0.5	1	(0)	-1	70	(47)	-23
	1.0 + 1.0	1	(0)	-1	55	(40)	-15
	1.5 + 1.0	1	(0)	-1	69	(46)	-23
dicamba + mecoprop + 2, 4-D....................	0.125+0.5+0.5	1	(3)	+2	57	(43)	-14
	0.125+1.0+0.5	0	(0)		64	(31)	-33
	0.125+0.5+1.0	0	(2)	+2	54	(37)	-17
	0.125+1.0+1.0	0	(0)		29	(26)	-3

[a]Adapted from the data of Jagschitz and Skogley (4).
[b]Expected responses for combinations are shown in parentheses following each observed response. The differences between observed and expected values also are shown by a plus sign to indicate synergism and a minus, antagonism.

The calculations involved in determining the expected response of one three-way combination from Table 1 illustrate the efficiency of formula V compared to formula IV. For example, using dicamba at 0.125 lb/A in combination with mecoprop at 0.5 lb/A and 2,4-D at 0.5 lb/A the expected response is calculated as follows using formula IV and the data in terms of percent weed control as originally reported by Jagschitz *et al.* (4).

$$E = 45 + 3 + 37 - \frac{[45(3) + 45(37) + 3(37)]}{100} + \frac{(45)(3)(37)}{10{,}000}$$

$$= 85 - \frac{(135 + 1665 + 111)}{100} + \frac{4995}{10{,}000}$$

$$= 85 - 19.11 + 0.50$$

= 66.39% weed control expected.

Using formula V and percent-of-control values, the computation is

$$E_1 = \frac{(55)(97)(63)}{10,000}$$

$= 33.61$ percent of control

and 33.61%-of-control is equal to 66.39% weed control.

Obviously, there are practical limitations in using mathematical formulas in predicting the responses for herbicide combinations. The methods described here are approximations, but they represent an improvement over no attempt to predict responses. The computations described should most effectively be applied to populations of single species although this would not seem to be an absolute requirement. Furthermore, the formulas are most accurate when values of X, Y, and Z are near the 50% level since the dose-response curves deviate least from linearity at the 50% level.

Literature Cited

1. Colby, S. R. and G. F. Warren. 1963. Herbicides: combination enhances selectivity. Science 141:3578.
2. Colby, S. R. 1965. Greenhouse evaluation of herbicide combinations. Proc. NEWCC 19:312-320.
3. Gowing, D. P. 1960. Comments on tests of herbicide mixtures. Weeds 8:379-391.
4. Jagschitz, J. A. and C. R. Skogley. 1966. Dicamba, mecoprop, and 2,4-D combinations for the control of clover, chickweed, and dandelion in turfgrass. Proc. NEWCC 20:496-501.
5. Limpel, L. E., P. H. Schuldt, and D. Lamont. 1962. Weed control by dimethyl tetrachloroterephthalate alone and in certain combinations. Proc. NEWCC 16:48-53.
6. Tammes, P. M. L. 1964. Isoboles, a graphic representation of synergism in pesticides. Neth. J. Plant. Path. 70:73-80.